CONTRIBUTIONS TO OUR KNOWLEDGE OF SOIL-FERTILITY.

v. *The Action of Fat-Solvents upon Sewage-sick Soils.*

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In a paper upon sewage-sickness in soil and its amelioration by partial sterilisation, Russell and Golding* discuss the causes of sewage-sickness with special reference to adverse physical and biological conditions. Certain physical conditions, such as the deflocculation of the clay by the alkali of the sewage, lead to a water-logging of the soil which enables certain large organisms to grow freely, while the bacteria that induce decomposition are injuriously affected. In addition to this, the large organisms prey upon the bacteria. With this combination of factors, the decomposition of the sewage stops, and the soil is said to be sick. Upon resting the soil, the excess of water disappears, the conditions become unfavourable to the larger organisms but favourable to the bacteria, and the soil again becomes fit to receive sewage. Extending the results obtained by them in the treatment of soils by volatile disinfectants, they claim that a partially rested sewage-soil, in which the protozoa have been destroyed by treatment with toluol or carbon bisulphide, is more effective as a filter than an untreated soil, and that the soil does not so quickly lose its efficiency.

In my work upon soils, I have shown† that the numerical increase of the bacteria is limited by the bacterial toxins and fatty substances which are found normally in soils. The fatty substances, which I have named collectively agricere, waterproof, as it were, the particles of nitrogenous organic matter, and prevent their rapid disintegration. When the soils are treated with the volatile disinfectants, which are also fat-solvents, the water-

* Journ. Soc. Chem. Ind. xxx. (1911), p.471.

† These Proceedings, 1910, p.808; 1911, p.679.

proofing is destroyed, and a freer passage is given to the diffusion of the nutrients. More nutritive material is at the disposal of the bacteria, and, as a consequence, there is an increased growth.

The case of sewage-sick soils appears, at first sight, to be of a different nature from that of ordinary soils, inasmuch as the sewage itself is a nutritive solution, and decomposition will occur in the sewage irrespective of the soil-particles. The sickness, however, is caused by the absence of decomposition, brought about by the waterlogged condition of the soil preventing the sewage passing through with sufficient ease to enable the soil to become aërated.

In efficient sand-filters and sewage-soils, the particles are covered with bacterial slime, and it is reasonable to suppose that, in the absence of clay, the excessive formation of this slime must tend to clog the filtering soil. But while the slime is growing, there is a gradual deposition of fatty matter from the soap of the domestic sewage, and, in time, the interstices of the soil become filled with a jelly of slime and fatty material, which checks the free passage of the sewage. From the accumulation of fatty material, sewage-sick soils should therefore be extremely sensitive to the action of the volatile disinfectants or fat-solvents.

When the sewage-sick soil is rested, the drier condition will doubtless enable the bacteria to decompose the slime, but the mere drying may be enough to modify its gelatinous nature. I have seen a slimy solution become quite limpid during evaporation. It was the case of a slime obtained from a bath-sponge, and, at the time, I thought that the limpidity had been caused by the slime combining with the lime-salts of the tap-water which had been used. A similar change may occur when sewage-sick soils are dried. Once they are thoroughly dried and moistened, they certainly do not become so tough in texture as before.

A sample of sewage-sick soil was obtained from the Metropolitan Board of Water Supply and Sewerage, by favour of the Medical Officer, Dr. E. S. Stokes. The soil had originally been a shelly sand such as is found upon the shores of Port Jackson, and, in the sample, the original nature of the filter-bed was clearly seen. The sewage had come from the residential suburb of North Sydney, and contained no trade-effluents. Furthermore, no clay was present, so that a deflocculation by the sewage was out of the question. The worms, etc., were picked out, and the sample exposed to the air. It dried slowly, the clots and lumps retaining the moisture for a considerable time. When dry, it was gently broken up and sifted.

Shells, paper and wood retained by No. 13 sieve	4·5
Coarse sand and shell fragments retained by No. 24 sieve	4·6
Fine soil	90·9

A portion of the soil (40 g.) was treated in the Soxhlet fat-extraction apparatus with chloroform, and the extract weighed, after which it was saponified and resolved into paraffins and fatty acids. The residual soil was then extracted with alcohol. Following this, the soil was mixed with water, and heated in the autoclave at three atmospheres' pressure for three hours; the supernatant solution was filtered off, evaporated, and the residual gum dried. The partial analysis of the soil was as follows*—

Moisture at 98°	1·43	
Moisture at 130°	0·44	
Chloroform-soluble	1·08	
Fatty acids		0·81
Paraffins		0·18
Alcohol soluble	0·69	
Fatty acids		0·56 (partly lost)
Volatile and organic matter	7·60	
Crude gum		0·87
Sand and ash	88·76	

The amount of the fatty substances is considerable, and constitutes 19% of the total organic matter, while the crude gum is 9%. One can readily understand how this soil, containing as it

* As a control upon the first analysis, 10 gr. of soil were treated for three hours with ether in the Soxhlet apparatus, and, after the ether had evaporated, the portion was extracted, for the same time, with alcohol, and, finally, with chloroform.

Ether extract	1·00%
Alcohol extract	1·21%
Chloroform extract	0·09%
	2·30%

does, so large a proportion of fat and slime in relation to its organic matter, should only permit the passage of water with difficulty.

In my experiments with soils, the action of the agricere was demonstrated by treating the soil with a volatile disinfectant, such as chloroform, and after the evaporation of the solvent, the layers of soil were tested for their capacity of producing bacterial growth. As the chloroform kills off all the protozoa, this disturbing possible factor is removed, and the influence of the chloroform in translating the agricere is shown by the nutritive powers of the layers of soil being different. The upper layers contain the bulk of the agricere, deposited there by the evaporating solvent. The agricere, however, is deposited upon the particles irregularly; there is not the uniform distribution which obtained previous to the addition of the solvent. Still, there is more of the impervious agricere in the upper layers than in the lower, and, as a result, the upper layers are less actively nutritive, and the bacteria grow more quickly in the lower. This method was used in testing the sewage-sick soil.

The air-dried, sewage-sick soil was wetted with chloroform, and after all odour had passed off, the layers were carefully separated. Ten grams of each were placed in small wide-mouthed bottles, and moistened with 4 c.c. of sterile water, which was carefully stirred into the soil with a stiff wire. The adhering soil was removed with a small tuft of sterile cotton-wool, which was dropped into the bottle. The soils were incubated for eight days at 28°, evaporation being minimised by the bottles being covered with a bell-jar.

Soil after evaporation of the chloroform.	Soil-bacteria in 0·0001 gr. after 8 days at 28°.
Top layer	19,600
Second layer	29,400
Third ,, ...	28,200
Fourth ,, ...	29,600
Fifth ,, ...	35,400
Bottom ,, ...	39,000

The greater growth of the soil-bacteria in the lower, as compared with the top layers, is a confirmation of the view that the fatty matters of the sewage-sick soil are translated with the solvent, and deposited near the surface. It is clear that the treatment of the sewage-sick soil with volatile disinfectant does more than destroy the so-called phagocytic protozoa.

In dealing with the action of the fat-solvents upon soils, it is a comparatively easy matter to eliminate the protozoa by heating the soil at 60° for 10 minutes.* When this is done, the action of the solvent upon the agricere becomes clearly demonstrated. In the following experiment, a portion of the air dried, sewage-sick soil was heated at 62° for 10 minutes. Part of this heated soil was treated with chloroform, and upon the evaporation of the solvent, the soil was thoroughly mixed. Portions, each weighing 10grm., of the not heated, heated, and heated and chloroformed soils were put into small bottles, and moistened with three c.c. of sterile water. On the twelfth day, a further two c.c. of water were added to each of the remaining bottles, which were then covered with a small bell-jar to minimise evaporation.

SOIL-BACTERIA IN 0·0001 GRM.

Sewage-sick Soil.	At start.	4 days.	12 days.	25 days.	39 days.
Untreated soil....	52	680	2,700	4,300	5,400
Soil heated at 62°	16	15,800	11,800	9,000	8,000
Soil heated at 62° and treated with chloroform..............	13	24,600	45,400	41,600	90,000

It may be that the heating at 62°, by killing off the protozoa, has enabled the bacteria to grow more freely during the first four days, but the subsequent decrease rather points to the heat

* After noting that the harmful protozoa, etc., are destroyed at 60°, Russell and Golding heated the sewage-sick soils to 98° for three hours. For the purpose of their argument, this excessive heating was unnecessary. The effect is to destroy the natural toxins, and, as has been pointed out by Pickering, Russell and Hutchinson, and myself, to develop heat-toxins. The presence of natural soil-toxins is denied by Russell and Golding, who say that there is no satisfactory evidence of any toxic substance in soils that are not acid. Since their paper was published, I have shown that tap water can extract from ordinary soils certain substances of the nature of bacterial toxins. These are not acid, and reduce the number of living bacteria purposely added to the extract.

having destroyed some of the toxin. The gradual levelling up of the numbers in the untreated and heated soils, as time went on, favours this view. The slow diminution of the bacteria in the heated soil cannot be explained otherwise than by the autointoxication of the growing bacteria. The chloroform-treatment has clearly produced a profound change in the soil, and in the absence of protozoa, this must be ascribed to the redistribution of the fatty matters of the soil, whereby the nutrients are made more available.*

It is evident that the action of the volatile disinfectants upon the sewage-sick soils is not, as Russell and Golding affirm, to destroy the phagocytic protozoa, so much as to bring about a segregation of the agricere in which such soils are specially rich. The amount of the gum and fatty material in these soils is enough to justify the old idea that the sickness is due entirely to the physical state whereby the easy percolation of water or sewage and of air are prohibited. Inferentially the protozoa, etc., have a very limited action, if any, in causing or assisting sewage-sickness in soils.

* The toxins are also made more available, and it becomes a question of the quantity of nutrients and of toxins liberated, as well as of the susceptibility of the bacteria to the toxins.